Prevention and treatment of infectious diseases have improved considerably over the last decades due to the widespread use of vaccines and anti-infectives as well as the development of infection control strategies. Nevertheless, infectious diseases still cause substantial morbidity and mortality world-wide. Respiratory tract infections remain a leading cause of severe illness and mortality. Gastrointestinal diseases are still among the most reported diseases in Europe, and represent a huge health problem in all parts of the world with deaths particularly in children younger than 5 years of age. Nosocomial infections and chronic persistent infections are steadily rising because of an increasing number of vulnerable patients due to older age, immunosuppression, and increasingly invasive diagnostic and therapeutic interventions.

Furthermore, in the past years a series of unexpected new infectious diseases emerged, among them many food-borne and zoonotic diseases (e.g. H1N1 influenza A virus epidemic in 2009, and the German EHEC epidemic in 2011), and have illustrated our vulnerability. Our knowledge about the animal and environmental reservoirs of these pathogens is rather low. Crucial virulence mechanisms are still not fully understood and treatment options are often unsatisfactory due to low effectiveness in the reduction of symptoms or antibiotic
resistance. Misuse and overuse of antibiotics in medicine and in the livestock industry has resulted in the selection of multidrug-resistant pathogens, which cannot be eradicated by commonly used antibiotic therapies. The situation is exacerbated through the deficit of effective drugs for many persistent viral diseases and the lack of successful prevention measures. Another difficulty in the control of bacterial and viral pathogens is that they exhibit a high degree of diversity. They show strong surface-antigen variations, can rapidly adapt to changing environments and hosts, and have a very complex pathogenesis. Based on these challenges this topic focuses on the in-depth characterization of the biology of bacterial and viral pathogens and host-pathogen interactions to gain a comprehensive understanding of the virulence mechanisms and the complex infection processes. The topic has the three following research aims:

**Infection control – Unraveling risk factors for infections**

We have only limited knowledge about (i) the distribution of pathogens in the environment, in animal and in human populations, (ii) the pathogen signatures that are associated with high virulence and infectivity, and (iii) the host risk factors that determine persistence, transmission and infection. To successfully prevent infectious diseases and to reduce the public health burden of disease outbreaks this new research task of the HZI has started to address pathogen-associated factors relevant for transmission, infectiousness, persistence and spread of pathogens. This includes the identification of factors that enable important pathogens to persist and proliferate in external reservoirs, to colonize and infect humans (endogenous reservoirs) and to cause disease. It also implicates pathogen profiling to identify outbreak strains, biomarkers and functional traits of particularly dangerous pathogens (e.g. drug resistance, toxins) required to develop molecular diagnostic tools which are important for rapid detection and early, targeted antimicrobial therapies. Furthermore, conditions that determine the persistence of the pathogens in humans (e.g. certain changes of the composition of the microbiome and special colonization factors) are elucidated. This knowledge can be helpful as predictive markers for the disease outcome and for treatment decisions including dietary, lifestyle and/or pharmacological interventions.

<table>
<thead>
<tr>
<th>Nosocomial Infections</th>
<th>No. Infected people</th>
<th>No. Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>~ 2.000.000</td>
<td>48-99.000</td>
</tr>
<tr>
<td>Europe</td>
<td>&gt; 3.000.000</td>
<td>40-100.000</td>
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</table>
Infection strategies – Understanding molecular mechanisms of virulence

Viral and bacterial pathogens evolve rapidly and display different pathogenicity mechanisms leading to variable clinical manifestations which complicate development of effective antimicrobial drugs. Moreover, environmental and host factors influence host-pathogen interactions through diverse regulatory networks and iterative responses on both sides. Therefore, a comprehensive portrayal of viral and bacterial infection processes, reflecting the dynamic and complex nature of host-pathogen interactions at the system level, is required for this endeavor. One major aim of this research theme is to identify pathogenicity factors and virulence strategies, which can serve as targets for new therapeutic strategies or as antigens for protective vaccines among the complex set of virulence-associated traits. For this purpose virulence mechanisms crucial for i) the initiation of the infection process, including host cell adhesion and invasion, ii) proliferation within the host and iii) establishment of persistent/chronic infections are dissected at the molecular, functional and structural level. A key focus has been the elucidation of bacterial and viral host cell association and invasion mechanisms. Several novel cell adhesion molecules have been identified and interaction with host cell receptors and extracellular matrix proteins were characterized. In addition, microbial subversion of intracellular functions to facilitate host cell entry and transcytosis or to prevent uptake by professional phagocytes are studied. In general, multiple signalling pathways and/or several steps of certain signalling pathways, in particular those controlling rearrangements of the host cell cytoskeleton were found to be targeted by microbial factors from enteric pathogens and streptococci. Qualitative proteome and phosphokinome analyses determining the phosphorylation status of cell signalling molecules were established and used to unravel the spatial and temporal dynamic of microbe-triggered signalling processes. A major line of our research was also devoted to study complex regulatory networks implicating sensory and regulatory RNAs and microbial adaptation processes during different stages of the infection (e.g. host-adapted metabolic processes, immune escape mechanisms). These efforts provided a deeper insight into the molecular pathways of infectious diseases and highlighted novel opportunities for development of targeted therapies.
Infection intervention – Novel drug targets and anti-virulence strategies

Traditional antibiotics, which are widely used to combat bacterial infections, induce bacterial lysis or substantial stress, which rapidly selects for resistant subpopulations and can cause undesirable changes of the microbiota leading to a loss of symbiotic benefits. In parallel, directly acting antiviral drugs are challenged by high mutation and replication rates, which pose a substantial challenge for controlling virus replication and resistance. One compelling strategy to classical antimicrobial therapies is to inhibit crucial virulence strategies. This approach aims to find and target the weak points (the Achilles’ heel) of a pathogen by inhibiting fundamental pathogenicity mechanisms that initiate the infection, promote persistence and/or cause disease symptoms. This includes compounds that interfere with classical virulence factors such as adhesins/invasins, toxins, master virulence regulators and immune-modulators. Furthermore, establishment of advanced animal models coupled with novel imaging, ‘omic’ technologies and in vivo RNA-seq allowed us to initiate integrative analyses of the complex infection process which will help us to identify novel pathogenicity factors that may serve as potential drug targets. Several teams have started to exploit the knowledge of crucial virulence mechanisms and have developed test assays and high-throughput screening systems towards anti-virulence based drug discovery. Promising candidates of Hepatitis C virus-specific inhibitors and a Streptococcus biofilm damaging natural compound have been identified, and many more are expected to be discovered in the near future.
Virulence Factors of Streptococci and Pneumococci

Streptococcus pyogenes, S. dysgalactiae ssp. equisimilis (SDSE) and S. pneumoniae cause a wide spectrum of acute infections in humans and a severe autoimmune sequela named rheumatic fever. Morbidity and mortality due to streptococcal infections and their sequelae remain very high. We are elucidating the pathogenicity mechanisms in order to develop novel control strategies.

PARF is a highly relevant trigger of rheumatic fever
Peptide associated with rheumatic fever (PARF) is a collagen binding motif of S. pyogenes and SDSE. Moreover it causes the collagen IV autoimmunity in rheumatic fever triggering the autoimmune disease. We have investigated the epidemiological relevance of the PARF motif and showed that about 7% of the S. pyogenes and SDSE isolates that cause infections worldwide harbor an active PARF motif.

The correlation of SDSE with rheumatic heart disease is a cause of concern, because carriage of and infections with SDSE are often inadequately treated. We observed that more than 10% of the SDSE isolates from a high incidence region of rheumatic fever had an active PARF motif that leads to autoimmune reaction. This neglected streptococcal species must be taken seriously and should be eradicated.

Vaccines against streptococci
In many regions of the world, antibiotic treatment alone has proven insufficient in prevention of rheumatic fever. Improved diagnosis using our knowledge about PARF and development of a vaccine against the causative streptococci are two important strategies to fight this disease. M protein based vaccines are protective but bear the risk of causing autoimmunity as a side effect. We have identified the streptococcal arginine deiminase and trigger factor as protective antigens and 52 other potential candidate antigens for the development of safe alternative vaccines against pyogenic streptococci.
Streptococcal cell invasion and its consequences

The ability of streptococci to invade host cells protects them from the immune system and from antibiotic treatment. This leads to recurrence of infection and contributes to invasive infections. Streptococci disseminate via the blood circulation and invade endothelial cells through a phagocytosis-like process.

We have shown that FbaB (fibronectin binding protein of group A streptococci, type B) contributes to S. pyogenes invasion into endothelial cells (Fig. 1). Uptake into host cells was accompanied by formation of membrane protrusions with massive actin accumulation. Intracellularly, bacteria trafficked along the classical endocytic pathway ending up in phagolysosomes. FbaB is the first identified endothelial cell invasin of S. pyogenes.

We observed that adherence of Streptococcus pneumoniae to primary human pulmonary microvascular endothelial cells (HPMEC), in combination with sublytic concentrations of pneumolysin, stimulates the exocytosis of Weibel-Palade bodies (Fig. 2) and the secretion of von Willebrand factor and interleukin 8. In conclusion, S. pneumoniae induces release of proinflammatory and procoagulative components directly contributing to pathophysiological processes that lead to fatal tissue injury.

Our research provides new insights into the pathogenesis of streptococcal infections and sequelae. Furthermore, it delivers valuable information for translation into better diagnostics, vaccines and treatments.

Fig. 2. A) Uninfected endothelial cells (HPMEC) exhibit a high number of Weibel-Palade bodies (green). B) After infection with S. pneumoniae Weibel-Palade bodies are released to the outside; note the absence of green staining in the infected (S. pneumonia in pink) HPMEC cells. Photo: HD, Rohde

Publications


Project Members

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Enteropathogenic *Yersinia* species, *Y. pseudotuberculosis* and *Y. enterocolitica*, are faecal-oral zoonotic pathogens, which are ranked as pathogens of high importance in Germany. They colonise the intestinal tract and cause a large variety of gut-associated diseases in humans, ranging from diarrhoea, enterocolitis, and terminal ileitis to mesenteric lymphadenitis, that are collectively called yersiniosis. The diseases are typically self-limiting, although, sequelae such as reactive arthritis, erythema nodosum and thyroiditis are also common.

Yersiniae are widely distributed and contaminated pork is the main source of infection for men. They express a set of special surface structures which allow them to bind and internalize into host cells. These outer membrane proteins (adhesins and invasins) promote efficient attachment to cell receptors, which results in the induction of certain signal transduction pathways within the cell. Recruitment and activation of certain signal molecules to bacteria bound cell receptors induce rearrangements of the actin cytoskeleton and lead to the formation of special membrane protrusions which migrate around the bacteria and enclose it into a membrane-bound phagosome. Cell invasion allows transcytosis of the bacteria through the gut epithelium, into the underlying lymphatic tissues and deeper organs. We focus on the characterization of the function and expression of *Yersinia* invasion factors to gain more information about how enteric bacteria colonise host tissues and become resistant against the host immune system.

**Molecular analysis of the *Yersinia*-induced signal transduction in epithelial cells**

In order to identify signalling pathways which are essential for the internalization of *Yersinia pseudotuberculosis*, we studied the cell uptake promoted by the *Yersinia* invasion factors InvA and YadA which interact directly or indirectly via extracellular matrix proteins with β₁-integrin receptors of the host cell. By co-localization studies and kinase activation assays, we could demonstrate that the protein kinase B (Akt), the phospholipase C-γ and variants of the protein kinase C are recruited to bacteria-bound membranes and are activated in a time-dependent manner. Application of inhibitors and use of knock-out cell lines and RNA interference demonstrated that activation of these factors occurs after activation of the integrin-bound focal adhesion kinase (FAK), c-Src and the
PI3 kinase, and are required for the bacterial entry process. Furthermore, we obtained evidence that, besides the small GTPase Rac-1, also other GTPases of the Rho family and different actin-associated proteins, such as N-WASP and the Arp2/3 complex, are implicated in the uptake process. Furthermore, we characterized two newly identified adhesins of \textit{Y. pseudotuberculosis} with high homology to the invasin protein, and found that both promote tight binding to human enterocytes. First analyses in the mouse infection model revealed that loss of the adhesins prolonged the life time of infected mice significantly.

**Temperature-dependent expression of \textit{Yersinia} virulence factors**

Another important goal is to understand the molecular control mechanism of \textit{Yersinia} virulence genes during the infection process. Temperature is one of the most crucial factors sensed by the pathogens to adjust expression of their virulence factors after entry from a cold external environment into a warm-blooded host. We found that the regulator protein RovA controlling expression of the invasin protein undergoes a reversible conformational change upon a temperature shift from 30°C to 37°C. This reduces the DNA-binding affinity of the regulator and renders it more susceptible for proteolysis by the bacterial protease Lon. In addition, other post-transcriptional regulatory mechanisms were analyzed. These control systems are used to adjust virulence gene expression during the infection process to availability of nutrients and stress superimposed by the host immune system.
Viruses are intracellular parasites. They usurp cellular factors and machineries in order to propagate their viral genome and to release novel progeny virions for infection of new host cells. In the course of this project, we searched for cellular factors that facilitate release of infectious hepatitis C virus (HCV) particles.

HCV, like also influenza A viruses or Coronaviruses, belongs to the group of enveloped viruses. These viruses have in common that they wrap their viral genome contained in a protein shell with a lipid and protein rich membrane layer. This lipid membrane is acquired in a complex process at the surface of infected cells or within the cell at intracellular host membranes. Subsequently, the enveloped mature virus particles are liberated to meet and infect now target cells. If the virus envelope does not have the proper composition, these viruses are unable to infect new cells and the chain of infection events is interrupted.

**Results**

To identify novel host factors required for the assembly of HCV progeny, we used an alternative approach. Rather than manipulating individual host factors, we interfered with central signalling cascades of HCV host cells. Such
cascades regulate abundance and activity of a large number of cellular factors. Consequently, this strategy permits the blockade of specific host functions thus revealing a possible dependence of the virus on these specific cellular pathways.

Using this approach, we observed that a blockade of the mitogen-activated protein kinase pathway (MAPK-pathway) inhibited HCV propagation. Further molecular analyses revealed that the cellular cytosolic phospholipase A2 (PLA2G4A), an enzyme which is activated by the MAPK-pathway, is crucial for assembly of infectious HCV particles. At the same time, this enzyme plays an important role to initiate inflammatory reactions within cells. Specifically, PLA2G4A is recruited to the ER where it cleaves defined membrane lipids. This cleavage liberates a specific fatty acid, the so called arachidonic acid, which serves as building block for the synthesis of a number of inflammatory mediators. Moreover, the local cleavage of membrane lipids by PLA2G4A modifies the curvature and fluidity of these membranes.

Using inhibitors of PLA2G4A we were able to show that blocking this enzyme results in the production of aberrant HCV particles with disturbed membrane composition. As a consequence, these particles are poorly infectious. Interestingly, exogenous addition of arachidonic acid, the cleavage product of the enzymatic activity of PLA2G4A, restored production of infectious HCV particles suggesting that membrane associated arachidonic acid plays an essential role for the production of fully infectious HCV particles.

Remarkably, we observed that blocking of PLA2G4A not only inhibits HCV but also the related Dengue Virus that also assembles infectious particles at intracellular membranes. In contrast, the vesicular stomatitis virus, an animal pathogen that produces viral progeny at the cell surface, was not inhibited. These findings suggest that besides HCV and Dengue Viruses, other viruses that also assemble at intracellular membranes may depend on this cellular enzyme.

Therefore, we are now exploring if use of PLA2G4A is common to other human pathogenic viruses that use intracellular membranes to assembly infectious progeny (e.g. Coronaviruses). In parallel, we are testing other small molecules known to inhibit PLA2G4A. Some of these compounds are developed by pharmaceutical companies as inhibitors of inflammatory diseases. In the long run we aim to find out if manipulation of PLA2G4A function is a possible antiviral strategy for hepatitis C and other viral diseases (e.g. Dengue fever). A patent application that secures PLA2G4A as target for development of antiviral therapies against flaviviruses was recently filed.
Mechanisms of Host Defense Against Infection with *Staphylococcus aureus*

*Staphylococcus aureus* is one of the leading causes of both community-associated and nosocomial infections worldwide. It has developed resistance to a wide range of antibiotics, which complicates the treatment of infections. In particular, methicillin-resistant *S. aureus* (MRSA) has become a worldwide problem. Therefore, new therapeutic options with novel modes of action that bypass the development of resistance are required to tackle the problem posed by *S. aureus* infections in a more effective way. In this regard, therapeutic approaches aimed to enhance the efficiency of the host immune response to eliminate *S. aureus* may represent the best choice. However, in order to understand how the immune system can be manipulated to achieve a more efficient control of infection, we first need to obtain a clear understanding of how the host’s immune system responds to *S. aureus*. To achieve this understanding is one of the key objectives of the Infection Immunology Research Group.

*Dendritic cells are central coordinators of the host immune response to *S. aureus* infection*

Dendritic cells play an important role in the integration of the immune responses induced by pathogens. Using a mouse model of staphylococcal bacteremia, we have investigated the importance of dendritic cells in host defense against *S. aureus*. We found that depletion of dendritic cells resulted in substantial worsening of pathogen clearance and in accelerated mortality of *S. aureus*-infected mice (see Fig. 1). An additional finding of this study was that the beneficial role afforded by the dendritic cells during *S. aureus* infection might be mediated, at least in part, by the production of IL-12 since the detrimental effect of dendritic
T cells are critical for the control of *S. aureus* infection

Long-lasting protective immunity against pathogens depends on B and T cell mechanisms. As little is known about the adaptive immune responses to *S. aureus*, we here investigated the importance of B and T cells for the control of *S. aureus* during *in vivo* infection. Using different experimental approaches including a whole genome microarray combined with histology and flow cytometry, we have provided unequivocal evidence that the adaptive immune system is critical for the containment of *S. aureus* infection (see Figure 2). This postulation was confirmed by the demonstration that B and T cell-deficient RAG2−/− mice were less capable of restraining bacterial growth than immunocompetent mice. Most importantly, reconstitution with T but not with B lymphocytes significantly improved the capacity of RAG2−/− mice to control *S. aureus* indicating that T cells rather than B cells were involved in this process. Our results demonstrated that T cells and specifically CD4+ T cells are critical for controlling *S. aureus* infection. Therefore, CD4+T cell epitopes would be ideal targets for the inclusion in a *S. aureus* vaccine.

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**Fig. 2.** T cells are recruited into the site of infection in *S. aureus*-infected mice. (A) Immunostaining of a *S. aureus* infected kidney section showing the infiltration of CD3+ T cells (brown-stained cells). A kidney section from an uninfected animal stained for CD3+ T cells is shown in B. Original magnification, ×20 (A) and ×40 (B). Figure: HZI
Metabolic Diversity

Bacteria living in complex communities at scales that outnumber human cells colonize the human body. While these host-associated microbes are beneficial for human health, niches on the human body are also reservoirs for opportunistic pathogens. As an example, the human anterior nares are the major reservoir and thus risk factor for invasive infections by *Staphylococcus aureus*, an increasingly multi-resistant pathogen causing a large spectrum of infectious diseases.

To this end, we surveyed for the prevalence and molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in nursing home residents in Braunschweig (Germany), as nursing home residents are a population at risk for carrying meticillin-resistant *Staphylococcus aureus* (MRSA).

Among the 32 participating nursing homes of the available 34 in the region, 68% of residents (1827 of 2688) were screened for nasal and/or wound colonization. A total of 139 residents (7.6%; 95% confidence interval: 6.4–8.8%) were identified as MRSA positive, almost six-fold more than the 24 MRSA carriers (0.9%) expected according to the nursing homes’ pre-test information. Although known risk factors including urinary tract catheters, wounds, preceding hospital admission, and high-grade resident care were confirmed, none was sensitive enough to be considered as the sole determinant of MRSA carriage. Spa typing revealed that more than 70% of isolates belonged to the Barnim strain (ST-22, EMRSA-15, CC22) typical for hospital-acquired MRSA in Northern Germany. There was no evidence for the presence of community-acquired or livestock-associated *S. aureus* strains. This data shows that in Northern Germany MRSA has spread from the hospital environment to other healthcare institutions, which must now be regarded as important reservoirs for MRSA transmission.

Also, our knowledge on the composition of the nasal microbiota as a whole has increased substantially over the last couple of years, particularly as we have employed the newest of high-throughput methods allowing us to sample even more volunteers than before. Firstly, we sought to analyze a greater cross-section of the population by sampling 100 volunteers in Northern Germany. After optimizing and validating the method of terminal restriction fragment length polymorphism (TRFLP) against six previously pyrosequenced samples,
abundant species could be discriminated and their relative abundances measured in a high-throughput manner. The 100 volunteers could be statistically clustered into 12 groups, where two-thirds of volunteers shared more than 40% similarity in respect to their bacterial community structure, while the remaining third clustered into smaller groups being dominated by *Dolosigranulum pigrum*, *Moraxella spp.* or *Staphylococcus aureus*. *Moraxella spp.* was present predominantly in women rather than in men. Then, we assessed the temporal dynamics and variation of the global nasal bacterial community across 25 healthy volunteers (from the Braunschweig area) over 15 months. Overall, there was a global seasonal shift in bacterial community structure. Such a temporal shift was also strongly evident in the abundances of species such as *Propionibacterium acnes* and *Staphylococcus epidermidis*. However, such species dynamics over time was also inter-individual-dependent, and both individuals with highly stable communities and those with highly flexible communities could be defined. Even though the bacterial community of individual volunteers was generally variable over time and permanent carriage of a given species was seldom observed, various species - previously defined as constituting the core bacterial community - could be identified as persistent in a subset of the volunteers suggesting that these same species also constitute to a 'temporal' core community.
Biofilm Communities

The Project recognises symbiotic communities of micro-organisms in biofilms as functional units and pursues the goal of finding new pathways to control them by means of investigation of the diversity of microbial species and their interaction. The primary focus here is the fact that bacteria, in a natural setting, do not in most cases live as pure strains; rather, they live in communities. It is particularly interesting how pathogenic bacteria behave in the host and how they interact with non-facultative or facultative pathogenic bacteria in biofilms within the human body.

Development of dental biofilms

A considerable complication in the clinic is the infection of implants that are usually associated with the formation of biofilm on the implant. Such biofilms are difficult to combat since bacteria in biofilms feature special protective mechanisms against antibiotics and the immune system. Problems emerge particularly within the oral cavity, which by nature exhibits a plethora of various bacteria. To find out which bacteria occupy implants in the oral cavity, we examined two-week-old biofilms from various patients’ dental-implants. We found complex communities of bacteria in each case. When one compares these bacterial communities with those of infected implants, it becomes clear that infected implants exhibit a much larger quantity of different bacteria than...
healthy implants. We were able to show through statistic analysis that the bacterial communities in infected implants are quite characteristic for the particular patient; this is however rarely the case with healthy implants. Furthermore, it is interesting to note that the biofilm communities are apparently dependent upon the position of the implant within the mouth, for example incisors exhibit different bacteria compared to molars.

Metabolic activities in biofilms
The awareness of which bacteria appear where in which biofilms, is however only the first step towards understanding the complex phenomenon of biofilm. Substrate-uses on the part of individual bacteria within the biofilm community are also an important factor. In order to clarify how quickly and how comprehensively a substrate is inundated with bacteria, we implement substrates that are marked with stable isotopes and track their formation into the individual species of bacteria. Thus we can determine kinetics and modulate the flow of substances within the community (see figure). In order to facilitate clarification of not only interactions of bacteria together, but also interdependencies of host-bacteria, we began conducting such studies in cell-cultures and/or animal models. Since this involved non-radioactive materials, no special safety measures were required. We discovered another interaction between bacteria and host when we exposed the body’s own anti-microbial peptide defensin to *E. coli*. The bacteria subsequently produced adenosine, about which is known that it modulates the immune response. This could be significant for the treatment of infectious intestinal diseases.

Natural substances for the control of biofilms
It is the goal of these examinations to modulate biofilm communities and to expel the pathogenic bacteria from them. We utilize natural substances for this purpose but are also looking for new substances in fungi. Our initial investigation of fungi-isolates revealed that a series of quite differentiated natural substances is quite capable of dissolving biofilms of pathogenic bacteria, without functioning as an antibiotic. We found links from the class of the roridines, which are evidently able to inhibit, quite-specifically, certain fungi. Unfortunately, many of these compounds are also cytotoxic, so that the effective spectrum had to be carefully chosen. A small molecule that we named comatus-lacton is capable of dissolving biofilms of pathogenic bacteria, without acting as an antibiotic and without exhibiting such toxicity.
Pathogenesis of Chronic
*Pseudomonas aeruginosa* Infections

The diagnostic and therapeutic strategies that have served us well in the treatment of acute bacterial diseases have not yielded favorable outcomes when applied to chronic infections where bacteria grow in matrix-enclosed sessile biofilm communities. Although every single cell is able to induce a stress response with a characteristic change in the expression pattern, living within populations provides a species with additional mechanisms of survival, the most obvious one being heterogeneity and cooperation.

**Diversity facilitates survival**

*Pseudomonas aeruginosa* is the most dominant bacterial pathogen causing chronic lung infection in cystic fibrosis (CF) patients. Although most patients are colonized only with one or few *P. aeruginosa* clones, the isolation of various morphotypes is a very characteristic microbiological finding. This diversity seems to play a key role in the persistence of chronic lung infections. Our research focusses on the elucidation of the molecular mechanisms responsible for this diversity and the characterization of particularly well adapted *P. aeruginosa* biofilm phenotypes.

*Phenotypic variability of biofilm-grown P. aeruginosa isolates. Easy3D-projections represent 48h old biofilms of 15 clinical P. aeruginosa isolates and the lab strain PA14 stained with the BacLight bacterial viability kit. Viable bacteria are stained in green (Syto 9), dead bacteria in red (propidium iodide). Figure: HZI*
Small colony variants
We have previously demonstrated that an adherent sub-group of so called small colony variants (SCVs) is selected in the CF lung. By applying whole genome sequencing approaches we have identified mutations that could be associated with increased intracellular c-di-GMP levels and thus were demonstrated to be responsible for the switch to an auto-aggregative SCV phenotype. The identification of genotypes that are specifically selected at different stages of chronic infections will significantly advance our knowledge on the evolution and adaptation mechanisms of P. aeruginosa to its habitat. In this context not only SCVs but also other bacterial phenotypes that evolve during persistent P. aeruginosa infections in the CF lung, such as antimicrobial resistant isolates and biofilm phenotypes, are of particular interest. Extensive phenotype-genotype correlation studies are performed to elucidate the genetic determinants of infection relevant phenotypes.

Cooperation is supported by communication
Apart from two homoserine lactones, P. aeruginosa produces a third intercellular signal that is referred to as the Pseudomonas quinolone signal (PQS). PQS is involved in cell density-dependent virulence factor regulation – also known as quorum sensing (QS) – and the establishment of biofilms. However, the molecular mechanisms underlying downstream PQS signalling are largely unknown. PqsE, encoded on the last gene of the PQS biosynthetic operon, seems to play a key role in the translation of the presence of the PQS signal into bacterial behavior at the single cell level. The elucidation of the function of this important enzyme is a major focus of the group.

In summary, the identification of molecular mechanisms and bacterial biomarkers that can predict disease outcome of P. aeruginosa infections is a major focus of the group. Knowledge of their identity might eventually lead to the establishment of molecular diagnostic test systems for a reporting of P. aeruginosa resistance and pathogenicity profiles and thus may impact on therapeutic strategies and mitigate the future potential to evolve resistance.
**Microbial Communication**

Secretion and detection of small chemical signalling molecules (termed quorum sensing) allows bacteria to adjust their physiology to the presence and density of their own and foreign bacterial species as well as to eukaryotic members of the community. It is important for biofilm formation and virulence and thus could be a new target for anti-infectives. We studied quorum sensing signalling cascades, developed antibacterial screens and investigated the mechanism of action of carolacton, a new biofilm inhibitor.

**Fratricide in the caries bacterium *Streptococcus mutans***

Streptococci regulate genetic competence through a small autoinducer called “competence stimulating peptide”. Genetic competence is the ability to take up external free DNA and integrate it into the genome. It requires a complex protein machinery in the cell membrane, termed transformasome. Interestingly, quorum sensing simultaneously induces death of a fraction of the population, which thus provides nutrients and genetic material for its siblings, a phenomenon called fratricide. We were able to sort the population of *S. mutans* into...
the induced and uninduced fractions using FACS (fluorescence activated cell sorting). To this end, a reporter strain was constructed which expressed the green fluorescent protein when quorum sensing was induced. The gene expression of the two different cell populations was analysed separately by microarray analysis and provided detailed insights into the regulation of competence and cell death.

**Carolacton, a novel inhibitor of biofilm formation in *Streptococcus mutans***

Carolacton is a secondary metabolite which is produced by the soil bacterium *Sorangium cellulosum*. It inhibits biofilm formation of *Streptococcus mutans* at nanomolar concentrations. A study of its mechanism using time-resolved microarray analysis and knock-out of potential molecular targets showed that a novel protein kinase is essential for carolacton action, the serine-threonine protein kinase PknB (protein kinase B). It is present in monocopy in Streptococci and regulates cell division, but also modulates signalling through the two-component systems. These are the main interfaces for environmental adaptation in Streptococci. *S. mutans* has fourteen of them. The most important ones are VrcRK, which is the only essential one (*i.e.* it cannot be knocked out), and ComDE, which is induced by quorum sensing. Both of them are strongly affected by carolacton. Thus carolacton interferes with a signalling cascade important for growth, virulence and quorum sensing in *S. mutans*. 

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**Publications**


Structural Analysis of Virulence Factors

Using X-ray crystallography we determine the atomic structures of biomacromolecules (like proteins or nucleic acids) involved in bacterial and viral infection processes and the biosynthesis of natural compounds.

**Virulence Factors in Bacterial and Viral Infections**

Pathogens often rely on thermosensing to adjust their virulence gene expression. In *Yersinia enterocolitica*, the transcriptional master regulator RovA controls its own activity by a built-in thermosensor. Inside warm-blooded hosts, RovA undergoes conformational changes that attenuate DNA binding making the protein prone to proteolysis. In cooperation with the Dersch group we solved the structure of free and DNA-bound RovA and found that a loop in the dimerization domain and residues in the adjacent C-terminal helix being prone to unfolding at 37 °C. This structural distortion is relayed to the flexible DNA-binding domain leading to the release of RovA from its operator sites and to RovA’s subsequent degradation. In contrast to RovA, SlyA, a close homologue from *Salmonella*, is active and stable at 37 °C. Adapting RovA to SlyA by site-directed mutagenesis results in the complete abolishment of RovA’s thermosensing properties.

Hepatitis C virus (HCV) NS3-4A protease is essential for viral replication. In cooperation with the Collins group we solved the structure of NS3-4A in complex with novel inhibitory peptides that have been obtained from phage display. The peptides bind to an alternative site in the protease via a novel “tyrosine finger” making them interesting for further HCV drug development. Due to a combination of geometrical constraints and to the impairment of the oxyanion hole the peptides resist cleavage by the active site. Optimization through combinato-
ional phagemid display and protein crystallography resulted in a 32-amino acid peptide that proved to be a potent inhibitor of viral replication in cell culture and was able to inhibit frequent resistance mutants of NS3-4A.

### Chemical Diversity of Natural Compounds

Polyketides are structurally diverse, medically important molecules showing various biological activities. During their biosynthesis, chain elongation uses diverse building blocks, the availability of which limits the variation of the polyketide side chains. Enzymes belonging to the crotonyl-CoA carboxylase/reductase (CCR) class can, in theory, form building blocks with any side chain from unsaturated fatty acid precursors. In cooperation with the Müller group we determined the first crystal structure of a CCR, the hexylmalonyl-CoA synthase CinF from *Streptomyces*, in complex with its substrate. Structural and biochemical analysis of CinF revealed how primary metabolic CCRs can evolve to produce new building blocks and set the stage for the design of altered polyketides with new properties (s.also Highlights: Review by Dirk Heinz & Rolf Müller "New options for rational biosynthetic engineering").

Tetrapyroles are among the compounds most crucial for life on Earth. In a long-standing cooperation with D. Jahn and J. Moser (TU Braunschweig) we study the structure of enzymes involved in heme and chlorophyll biosynthesis. Multi-subunit DPOR is a nitrogenase-like enzyme that catalyzes the two-electron reduction of protochlorophyllide a. It thereby alters the absorption properties of this molecule and provides the basis of photosynthesis. We solved the structure of substrate-bound DPOR trapped in transition state. Our analysis permitted investigation of the dynamic interplay between the DPOR subunits and led to a deeper understanding of electron transfer mechanisms of multiprotein complexes.

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**Project Members**

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**Fig. 3.** Left: Domain organization of the CinF monomer. Right: Comparison of CinF active site with those of other CCR’s (modeled) illustrating the criterion for side chain size of the building blocks synthesized.

**Figure: HZI**

**Fig. 4.** DPOR holo-complex with protochlorophyllide a and ADP-aluminium fluoride.

**Figure: HZI**

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**Publications**


Structural Biology of Viral Persistence and Mammalian Prions

Viruses are obligatory intracellular parasites and rely on cellular resources for their propagation. Viruses that remain in the host for a prolonged period of time face particular challenges. Their survival and replication depend on a fine balance between opsonized cellular pathways and resources to produce viral progeny. For example, the life-cycle of herpes viruses is characterized by two phases: lytic replication and latency. The cellular machinery is then hijacked to replicate the viral dsDNA episome. The molecular structural mechanism that controls herpes viral latency and also the interactions with host-derived co-factors might provide useful targets for antiviral therapeutic intervention.

In contrast to viruses and viroids, the prions are a unique class of pathogens, which are thought to propagate exclusively as self-templating protein conformations. In the associated human and non-human infectious neurodegenerative diseases, the mammalian prion protein PrP \(^\text{C}\) switches its conformation into an aggregated transmissible prion state, PrP \(^\text{Sc}\). Once a prion is introduced into a susceptible host, it triggers a PrP conversion cascade, which leads to prion disease (Fig. 1A). Understanding the underlying molecular mechanism of prions might provide conceptual advances to understand other forms of human neurodegenerations, including Alzheimer’s disease and Parkinson’s disease.
Herpes viral persistence

Kaposi’s Sarcoma Associated Herpes virus (KSHV) is a γ2 herpes virus that infects endothelial cells and B cells. It is associated with several malignancies. Murine herpes virus 68 (MHV-68) is a related γ2-herpes virus frequently used as a model to study the biology of γ-herpes viruses in vivo. The KSHV latency-associated nuclear antigen (kLANA) and the MHV68 mLANA (orf73) protein are required for latent viral replication and persistence. Latent episomal KSHV genomes and kLANA form nuclear microdomains, termed ‘LANA speckles’, which also contain cellular chromatin proteins. We solved the X-ray crystal structure of the C-terminal DNA binding domains (CTD) of kLANA (Fig. 1A) and MHV-68 mLANA. Opposite to the DNA binding site, both kLANA and mLANA CTD contain a characteristic lysine-rich positively charged surface patch, which appears to be a unique feature of γ2-herpes viral LANA proteins. Importantly, kLANA and mLANA CTD dimers undergo higher order oligomerization (Fig. 1A). Using NMR spectroscopy we identified a specific binding site for the host-derived ET domains of BRD2/4 on kLANA (Fig. 1B). Functional studies employing multiple kLANA mutants indicate that the oligomerization of native kLANA CTD dimers, the characteristic basic patch and the ET binding site on the kLANA surface are required for the formation of kLANA ‘nuclear speckles’ and latent replication. Similarly, the basic patch on mLANA contributes to the establishment of MHV-68 latency in spleen cells in vivo. In summary, our data provides a structural basis for the formation of higher order LANA oligomers, which is required for nuclear speckle formation, latent replication and viral persistence.

Prions

Interspecies transmission barriers have been observed to correlate with the amino acid sequence of PrP. On the other hand, multiple prion strains of the same PrP amino acid sequence have been identified that cause characteristic pathologies in one host species. We aim to investigate prion 3D structures in order to understand the transmission barrier between mice and hamsters at atomic resolution, and to understand the structural basis of prion strains in relation to prion host range. We employ a combination of solution NMR, solid state NMR, and other biophysical techniques in order to obtain initial structural information of these aggregates. A key technique in achieving our research goals is the conversion of isotopically labelled recombinant PrPC into PrPSc. Therefore, we have invented a novel technology for the in vitro generation of specific amyloid-like aggregated prion conformations starting from prion infected brain tissue. The approach might be extended for the basic investigation of other neurodegenerative disorders like Alzheimer’s disease and Parkinson’s disease.
Structural and Mechanistical Analysis of Functional Amyloids

Bacterial fibrillar adhesins with an amyloid-like fold have recently been identified as a novel class of adhesins that are expressed by many Gram-positive and Gram-negative bacteria. A study on biofilms from different habitats established that up to 40% of the bacteria present within these communities carry amyloid-like fibrils on their surface. They have been identified as virulence traits important for biofilm formation, interaction with host proteins and promoting survival in a wide range of conditions. Amyloid-like fibrils are ordered aggregates that have a high content of specific beta-sheet secondary structure. Long associated with human diseases such as Alzheimer’s disease, Parkinson’s disease and Creutzfeld-Jacob disease, the amyloid fold is now known to be formed natively by a large set of diverse proteins without toxic side-effects.

The major focus of our group lies on the determination of the structural and mechanistical basis of these functions for selected bacterial and fungal amyloids.

Tools for the structure determination of amyloid fibrils
The size and non-crystalline nature of fibrillar protein assemblies drastically restrict the use of established structural techniques. We therefore employ quenched hydrogen exchange measured by nuclear magnetic resonance (NMR) and solid state NMR to study the structure and function of this important class of proteins. Solid-state NMR is a new method to determine high-resolution structures of insoluble protein assemblies. We have therefore established this technique at the HZI and now possess a custom-made, unique set-up that is also suitable to study infectious protein samples. In addition, we develop biochemical tools to generate protein samples with selective isotope labelling patterns.

Curli: an amyloid coat that increases bacterial virulence
Curli is the major proteinaceous component of the extracellular matrix produced by Enterobacteriaceae such as E. coli and Salmonella typhimurium. Curli fibrils are involved in the adhesion to biotic and abiotic surfaces and the promotion of biofilm formation. They also interact with several host proteins, resulting in increased tissue penetration, inflammation and sepsis. In vivo, curli biogenesis is dependent on the nucleation of the major curli component, CsgA, by the homologous protein CsgB. In order to understand the features responsible for
the different functionalities of CsgA and CsgB, we have analysed the structures, aggregation kinetics and thermodynamic stabilities of the fibrils formed by both proteins. In addition, we analyze the structure and function of chaperones that are essential for curli biogenesis in vivo. A mechanistical understanding of curli biogenesis will allow us to identify new ways to interfere with fibril formation in order to reduce bacterial virulence.

**HET-s: a functional prion found in filamentous fungi**

Only a subset of amyloids are self-propagating prions. To understand the molecular determinants that govern the infectivity of an amyloid, we investigate the biophysical properties of the functional prion protein HET-s from the filamentous fungus *Podospora anserina*, and of a recently identified homolog from *Fusarium graminearum*. Despite limited sequence identity, the amyloid fibrils formed by both proteins can cross-seed each other, i.e. they are able to breach the species barrier. Using quenched hydrogen exchange NMR, we could explain this observation with a high structural similarity of the amyloid fibrils.

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**Publications**


Intracellular Trafficking, Survival and Persistence of Streptococci

*Streptococcus pyogenes*, Group A streptococcus (GAS), is the major cause of human streptococcal infections that range from uncomplicated to severe and life-threatening infections like necrotizing fasciitis. Besides GAS, also Group C and G streptococci (GCS, GGS), *S. dysgalacticae* subsp. *equimilis* are common in invasive infections, especially in elderly persons. A hallmark of all streptococcal infections is that after a first episode of infection some patients experience recurrent infections like tonsillitis or erysipelas episodes— the carrier status of streptococci. Over the last years growing evidence has accumulated that recurrent infections may be attributed to streptococcal subpopulations in the patient which i) remain bound to extracellular matrix proteins like collagen or ii) due to intracellular persistence, therefore, avoiding the effect of antibiotic treatment and the onset of the host immune system. For intracellular persistence, streptococci have developed a battery of proteins for adhesion and invasion into host cells.

**Invasion and survival mechanisms**

Fibronectin-binding proteins of streptococci play an important role in adherence and invasion. Especially the streptococcal fibronectin-binding protein I (SfbI) and the GGS fibronectin-binding protein A (GfbA) have been studied in greater detail. Caveolae-mediated invasion leads to trafficking into, and survival, in caveosomes whereas invasion via cytoskeletal alterations leads to trafficking alongside the classical endocytic pathway with lysosomal fusion to form phagolysosomes and a reduced survival rate. Image: Manfred Rohde
detail. Two major invasion mechanisms of GAS, GGS and other streptococci have been elucidated so far, i) invasion via cytoskeletal rearrangements by triggering signalling cascades in the host cell and ii) invasion via large invaginations formed by the fusion of caveolae mediated by SfbI. Our studies aimed to elucidate the trafficking pathways inside the host cells. The results revealed that all streptococci invading via cytoskeletal rearrangements follow the intracellular classical endocytic pathway with subsequent fusion with lysosomes to form phagolysosomes. Due to the binding of fibronectin to SfbI and subsequent binding to and clustering of integrins SfbI expressing GAS co-opt host caveolae and reside inside caveosomes. Therefore, they are preventing fusion with the cellular degrading mechanisms via lysosomes. This contributes to better survival inside the host cells in the cell culture model (see Fig. 1).

For survival of streptococci in nutrient broth only scarce information is available. To shed light onto possible survival times in nutrient broth we cultivated GAS in TSB broth for 45 months and examined the streptococcal morphology. As is evident from Fig. 2 the 45-months old streptococci were morphological identical to those grown overnight after inoculating TSB with 5 µl of the 45-months old streptococcal pellet when examined by scanning EM. Only ultrathin sections revealed major structural differences in the cytoplasm of streptococci between 45-months old and freshly grown TSB cultures. These results suggest that streptococci can persist for a long time inside the human body and are able to cause recurrent infections in very short times when the environmental circumstances for growth are optimal.
Molecular Diagnostics of Microbial Pathogens

Major infection routes for microbial pathogens from the environment are drinking water, food and air. Water and food are primary sources of diarrheal infections whereas air is the major source of respiratory infections. To cover all major environmental infection routes, specific parts of the environment functioning as reservoirs were considered and relevant pathogens were studied.

**Coastal marine environments as reservoir of Vibrio cholerae**

There is a considerable global effort to reduce the risk of Vibrio infections and yet in many countries, illnesses associated with these bacteria are increasing. We used historical samples from the continuous plankton recorder (CPR) archive in Plymouth, UK, to demonstrate that the genus Vibrio, including the human pathogen V. cholerae, has increased in prevalence during the last 50 years.
in the coastal waters of the southern North Sea. This increase was correlated significantly with increasing sea surface temperature during the same period. Global warming may have a strong impact on the composition of the marine bacterial community with important implications for human health.

**Environmental reservoirs of waterborne pathogens in drinking water**

There is a rather limited knowledge on the microbiological principles governing the prevalence of microbial pathogens in drinking water. Drinking water distribution systems (DWDS) provide two very different types of habitats for bacteria: bulk water and adjacent biofilms. Biofilms are present in every DWDS attached to the surface of tubing materials. We studied the bacterial core community of bulk water and corresponding biofilms of a more than 20 year old DWDS providing drinking water to the HZI. The structure and composition of the bacterial core community in the bulk water was highly similar across sampling sites whereas all biofilm samples contained unique communities with very little overlap with species from bulk water. All biofilm communities showed higher relative abundances of individual bacterial species and a reduced richness compared to bulk water.

An infectious disease with increasing relevance for developed countries is legionellosis which is freshwater-based but caused by inhaled aerosol droplets containing pathogenic *Legionella* species. Therefore, these species could also be regarded as airborne pathogens. We studied the occurrence of different *Legionella* species in cold and hot drinking water using genus-specific molecular fingerprints. We could demonstrate that the treatment of the raw water reduced some of the *Legionella* species and increased others. In hot drinking water *L. pneumophila* was abundant all year round whereas in cold drinking water it only occurred during winter.

**Airborne pathogens in cystic fibrosis patients**

Airborne pathogens cause by far the most relevant human infections with influenza pandemics being only the tip of the iceberg. The resulting infections are often characterized by successions of a primary viral infection followed by secondary bacterial infections. This polymicrobial nature makes these infections difficult to diagnose and treat in an appropriate manner. As an example for polymicrobial infections, we studied sputum samples from the cystic fibrosis (CF) cohort of the Medical School Hannover. These samples were analyzed by universal bacterial fingerprints leading to a comprehensive assessment of the bacterial content of the sputum. Interestingly, a single bacterial species often dominated the sputum and these dominant bacteria belonged to several pathogenic species. This universal bacterial detection was complemented by a universal fungal detection fingerprint. Interestingly, we could show that the fungal diversity in the sputum of the whole cohort studied was about twice as high (60 species) than the bacterial diversity. This combined approach for fungal-bacterial diagnostics now provides an efficient tool to obtain novel insights into polymicrobial lung infections.
Analysis of Protein Networks Induced by Early Host-Pathogen Interactions

The Research Group Cellular Proteomics focuses on the analysis of host signalling pathways involved in early events of infectious processes in humans and the host’s adaptive immune responses towards pathogens. Modules for cell biological, biochemical, mass spectrometric and bioinformatic approaches have been developed and combined for this purpose. Our aim: a quantitative and time-resolved characterization of novel potential drug targets and mechanisms of post-translational modifications (PTMs) in tissues as well as primary and immortalised human cells.

Quantitative peptide sequencing by accurate mass spectrometry facilitates the identification of cellular “target proteins” and the understanding of signalling networks. Immobilised small molecules are used as “baits” to enrich and analyse the activities of signalling components. These, combined with chromatographic and mass spectrometric methods, enable analyses of transient modifications on signalling components. In case of phosphorylations this refers...
to protein kinases, i.e. their activities and molecular interactions with substrate molecules. iTRAQ™-technology is used for quantitative peptide sequencing and iTRAQassist for statistical evaluations, both matching the quality criteria for clinical studies based on tissues and primary cells.

**MET signalling pathways during invasion by *Listeria monocytogenes***

The bacterial pathogen *L. monocytogenes* causes severe illnesses and prenatal infections. The virulence factor InlB interacts with the receptor tyrosine kinase c-Met and induces its endocytosis and thus the uptake of the pathogen into host cells. The signalling pathways involved are controlled through protein phosphorylations by kinases as well as (de)ubiquitinations. The first minutes of kinase-dependent signal transduction were analysed for this pathway for the first time. We identified signalling components involved in listerial invasion that were not described for the c-Met pathways activated by the physiological ligand, HGF. Functional investigations of these kinases thus determine their contribution to the invasion as well as to motogenic and mitogenic processes. Indications for counter-regulation of this invasion pathway were obtained from analyses of human ubiquitin proteases (DUBs) whose activities are influenced by Listeria.

**Signalling pathways involved in activation of NK and regulatory T cells**

T lymphocytes are essential for regulating the immune system. The different cellular responses of conventional and regulatory T cells depend directly on the differential activation of such pathways. Comparative phosphokinome analyses of primary regulatory T cells detected novel components of the CD3/CD28-dependent activation pathways and microtubule-regulating proteins. Mechanisms of proximal signal integration were also analysed in natural killer (NK) cells within 2 minutes after activation. NK cells are part of the innate immune response and interact through the immunological synapse (IS) with virus-infected cells. A donor-specific analysis of their maturation led to the identification of novel IS components. The characterization of general mechanisms guiding microtubule-dependent processes within the IS constitutes a new research focus. Of particular interest are investigations of the regulation of tubulin tyrosine ligase that coordinates the recruitment of intracellular motor proteins.

In summary, the elucidation of early signalling events in host endothelial and immune cells following interaction with pathogens will provide insights into regulatory networks leading to the identification of novel targets for therapeutic intervention.

**Publications**


**Project Members**

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Bioinformatics and Statistics: Statistical Evaluation of Functional Genomics and Proteomics Data

Cellular biomolecules are accessible by different high throughput technologies and are the target of systematic analyses in order to reveal the molecular workings of organisms, which is essential for understanding infection diseases and to find treatments against them.

Data analysis of high throughput experiments can provide novel and biological relevant hypotheses, but standard statistical approaches are often not suited for such data. The type (gene, RNA, protein, metabolite and number of molecular) of components contributing to the observed infection phenotypes are often unknown at the beginning of a project. We utilize methods from exploratory data analysis and data mining as well as corrections for multiple testing to handle such multivariate data sets and to reveal components actually involved in infection.

Standard statistical approaches fail to provide good models (blue curve) when a high proportion of noise is present (data on the left). Robust methods (green line) can handle such data better. This is an example from flow cytometry. Figure: HZI
Raw data from technologies like mass spectrometry, microarrays, next generation sequencing or imaging technologies are naturally corrupted by noise. We provide special methods and noise models to distinguish biological effects from random fluctuations, especially for proteomics experiments, but also for other types of experiments.

Although the data sets from high throughput experiments themselves can be large, in most cases, few repetitions per experiment are available, leading to problems with classical statistical methods often based on larger sample sizes. Thus, a careful design of experiment as well as suitable statistical techniques are needed to generate reliable hypotheses in functional genome studies. Various approaches based on robust statistics and Bayesian inference have been developed to cope with these problems.

In summary, suitable data analysis techniques and statistical methods are essential for the analysis and evaluation of data from high throughput experiments to generate and test hypothesis in a reliable way. Our recent and future work focuses on data from time series experiments where the dynamics of the concentration of cellular biomolecules is observed over a period of time.

Publications


Epidemiological Determinants for Viral and Bacterial Infections in Human Populations

Infectious disease research in humans carries the following imminent challenges: first, the risk to become infected is often dynamically linked to the extend by which other people in the environment are infectious; second, a large proportion of infections are inapparent; and third, infections may later result in non-communicable diseases. In order to overcome these challenges we are establishing special epidemiological methods to prospectively capture infections as they occur in healthy individuals. All our activities are intertwined with the project “Clinical outcomes of infectious diseases” (Mikolajczyk) and closely linked to the project “Molecular epidemiology of acute respiratory infections” (Pessler). Our joint attention is focussed on infectious diseases with high public health relevance.

Establishment of the National Cohort in Hannover/Braunschweig
We run one of 18 study centres of the National Cohort – the largest health research enterprise ever in Germany and one of the very few large cohort studies globally to simultaneously address infectious and non-communicable diseases. Our department coordinates all infection and immunity related issues in the National Cohort, in which a total of 200,000 participants will be re-examined periodically every 4 to 5 years for various decades. Our new study centre in Hannover has successfully completed the Pretest II in 2012.
We have already conducted several feasibility studies of special infection-related modules. One example is the self-collection of vaginal lavage by female participants to detect urogenital infections. This will enable us to study the effectiveness of a vaccine against human papilloma virus in preventing cervical cancer. After addition of further examination equipment and procedures, the National Cohort national pilot study will start in 2013.

Prevalence and persistence of Methicillin resistant Staphylococcus aureus (MRSA)
An increasing proportion of MRSA is being acquired not in the hospital but in the community and methillin sensitive *Staphylococcus aureus* (MSSA) may be causing a higher burden of diseases than previously thought. We therefore conducted a population-based study to measure prevalence of MRSA in the general population in Braunschweig and studied the persistence of MRSA-colonization in a longitudinal manner. We intend to expand this investigation to persons during, and after, hospital treatment and also to other cities in order to better understand the interaction between hospital and community-based MRSA and MSSA.

Helicobacter pylori (HP)
HP is associated with gastric and possibly with intestinal cancer. The epidemiology of HP is very variable and different international groups are working on vaccines against HP. Within, and complementary to, the National Cohort we initiated epidemiological studies to generate evidence on how vaccinations and other measures could reduce the burden of HP related disease. For this purpose we are currently conducting a joint project with the German Center for Cancer Research (DKFZ) to establish methods of multiplex serology related to HP, which we later intend to expand to further pathogens.

Infections and associated metabolic diseases
Recent research indicates an increasing number of links between infections and metabolic diseases. Since the proposed causality mechanisms often require a long delay between infections and their metabolic consequences, studies in prospective cohorts are necessary and the National Cohort will provide the perfect research infrastructure to address this challenge.
Regulation by RNAs and Proteins – Modulators in Bacterial Pathogens

A greater understanding of the fundamental mechanisms of regulation in pathogens is critical to generate new findings for possible translation into novel biotechnological (e.g. genome editing tools) and biomedical (e.g. anti-infective strategies) applications. The Department “Regulation in Infection Biology” investigates regulation in processes of infection and immunity with a focus on Gram-positive human pathogens. A successful example of potential application of our research in biotechnology and biomedicine is our recent discovery of a novel genome editing tool (dual-RNA programmable DNA cleavage enzyme Cas9) that stems from our analysis of the adaptive immune CRISPR-Cas system in bacterial pathogens.

In our department, we are particularly interested in deciphering how RNAs and proteins succeed to orchestrate and fine-modulate gene expression in bacteria at the transcriptional, post-transcriptional and post-translational level. We study regulatory RNAs and proteins in various biological pathways such as horizontal gene transfer, adaptation to stress, physiology, persistence, virulence, infection and immunity. A favorite pathogen in the laboratory is *Streptococcus pyogenes*, also called Group A streptococcus, that can cause highly aggressive invasive infections such as toxic shock and necrotising diseases. In past years, we have also been investigating the genetics and biology of *Listeria monocytogenes, Staphylococcus aureus* and *Streptococcus pneumoniae*. 
Regulation by small RNAs

To protect themselves from the acquisition of detrimental or beneficial genetic material (phages, plasmids), bacteria have evolved an RNA-guided adaptive immunity system, called CRISPR-Cas. We are deciphering the molecular mechanisms involved in the adaptation, expression and interference phases of the immune system and pursuing the detailed analysis of the recently discovered Cas9-tracrRNA:crRNA genome editing device. In addition to the CRISPR-associated RNAs, we are addressing the question how small RNAs and RNA-interacting proteins integrate into the general regulatory network controlling pathogenesis and related mechanisms in *S. pyogenes* at the molecular and cellular level.

Regulation by chaperones/proteases

During infection, bacterial pathogens face a wide variety of adverse and fluctuating conditions within the host and have evolved multiple strategies to mount appropriate responses. Clp (Caseinolytic proteins)/HSP100 (heat-shock proteins) proteins are important components of the bacterial stress response, influencing adaptation, survival or virulence. We have identified substrates of Clps in Gram-positive pathogens and are investigating the mechanisms of substrate targeting and degradation at the molecular and cellular level. We are also interested in the regulation of the clp response by arginine phosphoswitch.

RNA and protein modulators in innate immunity

Initial recognition of pathogens by the innate immune system constitutes the key step in defense against infectious microorganisms. An inappropriate recognition may result in insufficient immune responses, yet an over-activation of the immune system may be equally deleterious. Innate immune cells respond to *S. pyogenes* infection by producing pro-inflammatory cytokines in a manner dependent of the adaptor molecule MyD88. We are interested in investigating RNA- and protein-mediated mechanisms involved in the response of immune cells to *S. pyogenes* infection.

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Staphylococcus aureus is an emerging pathogen and a leading cause of nosocomial infections worldwide. It can induce a wide variety of infections ranging from mild skin diseases to life-threatening systemic infections such as bacteremia. The pathogenic diversity of S. aureus is mediated by a large set of virulence factors produced in different combinations in various isolates. In addition, it becomes more and more evident that specific metabolic traits and their regulatory systems are crucial for fitness and survival of the pathogen during the infection process and thus indirectly impact its virulence potential.

Physiological adaptation of S. aureus to different host niches
We established an online resource called Aureolib containing 4,692 time dependent protein synthesis profiles for 521 cytosolic proteins of S. aureus exposed to nine defined infection-related stimuli. The database can be used as tool for (i) functional predictions of so far uncharacterized proteins based on their expression kinetics, (ii) the integration of stimulons into regulatory networks and (iii) for showing signal transduction systems operating under defined and non-defined growth conditions. During colonization and infection, S. aureus...
is confronted with a multitude of signals including growth-limiting factors and life-threatening host defense mechanisms. Hence, adaptation of bacterial gene expression in natural habitats is a multi-signal response and it will be an important task to unravel this diverse network. Using Aureolib as a tool box, we are currently moving to these more complex, infection-relevant experimental models and we have started to analyze protein expression in S. aureus isolated from nasal secretions, milk and macrophages. This data will not only enhance our understanding of S. aureus infection biology, but will also decipher the process of nose colonization which is the main risk factor for nosocomial infections.

**Regulation and functional characterization of virulence factors in S. aureus**

Secreted proteins represent a reservoir of virulence factors. By dissecting the exoproteomes of 68 different clinical S. aureus isolates, we have demonstrated that, within a single bacterial species, the exoproteome composition can be extremely variable. Despite extensive efforts in functional characterization of virulence factors in S. aureus, the overall understanding of mechanisms for the pathogenesis of S. aureus infections is limited. This is also reflected by the fact that at least 60% of the secreted proteins are completely uncharacterized so far and were only derived from the genome sequence. In the S. aureus secretomes, 77 of these proteins have been identified indicating that they are really expressed. Functional characterization of these particular proteins is a challenging task for our future studies. Mutants of the corresponding genes, recombinant proteins and corresponding polyclonal antibodies are being generated and used for functional characterization. This includes binding to cells of the immune system and non-professional phagocytes, the response of these cells to the proteins, the humoral and cellular immune response, interaction with components of the innate immune system and virulence in animal models. By this way we want to get new insights into immune evasion mechanisms of S. aureus. An S. aureus protein array is being developed which will be used for detection of antibodies and interaction partners.
Actin-based Motility of Pathogenic Parasites

Malaria is a devastating worldwide health threat. Annually, over a million people die of malaria and up to 300 million get infected, mostly children and pregnant women. Malaria is also a significant economic burden, trapping the poorest areas in a downward spiral of poverty. Resistance to existing anti-malarial drugs is a growing problem, and there is an urgent need for new drug and vaccine candidates. Malaria is caused by Plasmodium spp., a group of unicellular, eukaryotic, intracellular parasites of the phylum Apicomplexa. These parasites use actin for both motility and host cell invasion. Their cytoskeleton differs significantly from that of higher eukaryotes and is, therefore, an attractive target for anti-malarial research. The parasite actin filaments are extremely short and their rapid turnover is regulated by a limited set of actin-binding proteins, which are poorly conserved with their mammalian homologues.

**Synchrotron-based structural biology elucidates mechanisms of disease**

We aim at an atomic-level understanding of the mechanisms, by which the malaria parasite uses its actin cytoskeleton for motility and host cell invasion, and seek ways to interfere with these processes. We use a combination of modern structural biology, including X-ray crystallography, small-angle scattering of X-rays, and electron microscopy, as well as complementary biophysical and biochemical methods to elucidate structure-function relationships of individual proteins and large regulatory complexes. We are also interested in the development of new synchrotron and free-electron laser applications for visualizing large cytoskeletal complexes at high resolution.
Plasmodium actin-binding proteins have divergent structures and functions

Plasmodium has only 10-15 actin-binding regulatory proteins; a strikingly small number compared to >150 in higher eukaryotes. The most important of these are profilin, formins, actin depolymerization factors (ADF), capping proteins, cyclase-associated protein, and coronin. These proteins have a conserved overall fold, but are poorly conserved at the sequence level and have somewhat divergent functions compared to the corresponding proteins from other eukaryotes.

We have determined the crystal structures of Plasmodium profilin and both ADF isoforms. Plasmodium profilin, despite structurally deviating significantly from all other profilins, has retained most of the biochemical characteristics of profilins. However, it has a significantly divergent actin-binding surface, rendering it an attractive drug target. Currently, we are characterizing the actin binding of Plasmodium profilin in more detail. Of the two Plasmodium ADFs, the minor isoform ADF2 has a conserved structure and biochemically resembles conventional ADFs. However, the predominantly expressed ADF1 lacks the filamentous actin-binding motifs. Plasmodium formins efficiently initiate polymerization also of the weakly polymerizing Plasmodium actin. However, at least formin 1 works independent of profilin unlike formins from higher eukaryotes.

To elucidate the atomic details of the proteins of interest, they are crystallized into microscopic, regular crystals, which diffract X-rays. Figure: Petri Kursula

Plasmodium formin 1 nucleates actin polymerization as dimers mediated by the lasso region in its FH2 domain.
Figure: Ignatev et al. 2012, PLoS ONE

Plasmodium profilin binds actin via a motif unique to apicomplexan profilins. This region of the protein is being investigated for inhibitor design purposes.
Figure: Kursula et al. 2008, Structure

Elucidation of the structure-function relationships in the regulation of actin dynamics will contribute to our understanding of the motility and invasion of the malaria parasite and eventually lead to new drug targets.

At DESY, we have unique possibilities to use the most modern synchrotron and X-ray laser facilities to answer outstanding questions concerning the biology of the most devastating infectious disease.

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Publications


Random and Targeted Genomic Integration of Viral Vectors, Generation of Humanized Liver Tissue in Mice

The interest in the development and use of humanized mice to investigate human biological systems in vivo outside the human body is continuously growing. Humanized mice are either transgenic animals expressing human genes and/or immunodeficient mice engrafted with human cells or tissues. Immunodeficient mice repopulated with human hepatocytes have already proven useful for the study of hepatitis virus life cycles and new antiviral approaches and various immunodeficient strains have emerged from different laboratories over time. We are investigating, how in vivo generation of human liver tissue in mice could be improved.

Macrophages play an important role in the rejection of xenogeneic cells and therefore represent a major obstacle to generating chimeric mice with human xenografts. The signal inhibitory regulatory protein α (SIRPα) receptor is a negative regulator of macrophage phagocytic activity and interacts in a species-specific fashion with its ligand CD47. Furthermore, SIRPα polymorphism in laboratory mouse strains significantly affects the extent of human CD47-mediated toleration of human xenotransplants. We could show recently that expression

BALB-ΔRAG/γuPA mice were transplanted with LV-Cd47-GFP transduced hepatocytes. The comparison of selection ratios reveals that the proportion of Cd47-expressing hepatocytes (green nodules) has doubled in the recipient mouse liver. Non-transduced human hepatocytes appear in red colour (immunohistology for human albumin). Overall, our findings strongly indicate a selective advantage for human primary hepatocytes ectopically expressing Cd47. Figure: HZI
of mouse CD47 in human hepatocytes significantly improves engraftment and repopulation of recipient mouse livers. Another line of research extends to the generation of human liver tissue in mice from renewable stem cell sources such as embryonic stem cells (ESC), induced pluripotent stem cells (iPS) or transprogrammed cells (iHeps).

In a second focus of research we study integration patterns of HIV virus vectors in the hematopoietic system and in the liver. All viruses, which integrate into the genome of a host cell, are capable of activating neighbouring genes and may transactivate oncogenes (“insertional mutagenesis”). Our studies utilizing retroviruses show organ-specific risk profiles for the development of clonal dominance and cancer. Our molecular and bioinformatics tools for the analysis of organ specific integrons will now be extended to other clinically relevant viral pathogens such as hepatitis B virus in clinical samples.

Our ongoing studies aim to increase the availability of humanized mouse models for biomedical research through innovative strategies by improving engraftment/repopulation of recipient mouse livers with human hepatocytes and by substituting primary cells by cell culture grown stem cells. Furthermore, we aim to delineate the role of viral genomic integration and risk of cancer development in the liver.

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